MAPPING ANATOMICAL CONNECTIVITY OF THE PRIMATE CEREBRAL CORTEX USING DIFFUSION MRI

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ABSTRACT

The human brain can be modeled as a small-world network that has the capability of handling complex cognitive function. However, that network as a whole still remains poorly understood partly due to a limited understanding of our non-human primate relatives' whole brain networks. A significant roadblock regarding non-human primate data is the lack of reliable cortical region labels, as almost all human whole brain network studies rely on such region-wise labeled brain masks, otherwise known as parcellation masks. The goal of this study is to develop a method to parcellate the cerebral cortex in primate brains independently of any pre-defined cortical region labels. This mask will then be used in future whole brain network analysis, where the connective white matter structures will be reconstructed between each region in the mask. For this study, derived methods were developed using data from our closest living primate relative, the chimpanzee. An initial method included the non-linear transformation of the human automatic anatomical labeling (AAL) cortical template to chimpanzee template space; however, structural and functional mismatches discredited that technique. The derived anatomically independent parcellation (AIP) method employed dynamic region growth of each node derived in the gray/white matter interface mask, and demonstrated high-resolution segmentation of the cortical regions over several inputted node numbers (200, 500, 1000, and 3000). Using the derived parcellation masks in network analysis, future applications of this technique in cross-species comparisons could help gain significant insight into the evolutionary changes in the organization of brain architecture, as well as the origins of some neurodegenerative diseases in both human and non-human primates.

CHAPTER 1: INTRODUCTION

HUMAN CONNECTIVITY NETWORKS

The complex nature of the human brain allows it to both efficiently generate and integrate information drawn from multiple sources. For example, auditory comprehension can be composed of both the heard speech and the seen movement of the source. These complex functions are performed through the communication between the functional (gray matter) regions in the brain. Connecting these functional areas are fiber bundles (white matter) composed of myelinated axons, which transfer electrical signals from region to region. An understanding of the architecture of the white matter network could reveal crucial information regarding the human brain; however, extensive studies into the architecture of these white matter connections have faced many challenges. Many of the techniques developed are invasive (e.g. post-mortem dissection), or do not provide holistic information regarding the brain network (e.g. functional MRI (fMRI/BOLD) studies). However, recent studies using technological advancements in diffusion magnetic resonance imaging (dMRI) have allowed the human brain white matter connections to be modeled as a network, which has provided crucial information regarding the relationships between structure and functionality (Hagmann, Cammoun et al. 2008; Honey, Sporns et al. 2009; Honey, Thivierge et al. 2010; Sporns, Tononi et al. 2005; Sporns, Honey et al. 2007).

DIFFUSION TENSOR IMAGING

Recent advances in the field of MRI have made research in human brain mapping more readily accessible and non-invasive. Diffusion MRI is an *in-vivo* imaging technique that measures the random (Brownian) motion of the water molecules in the brain (Basser, Mattiello et al. 1994). Processing of the data from these scans can provide quantifications of the relative degree and direction of water molecule movement in each voxel (i.e. volumetric pixel). This imaging technique has shown to have many applications in a wide range of areas, such as basic neuroscience, surgical planning, etc. (Hagmann, Kurant et al. 2007; Hagmann, Cammoun et al. 2008; Honey, Sporns et al. 2009; Honey, Thivierge et al. 2010).

Due to the highly anisotropic nature of white matter tissue, a three-dimensional image of the connective white matter fiber bundles can be reconstructed by piecing together discrete estimates of the underlying continuous fiber-orientation fields from the diffusion MRI data. This technique, known as tractography, has allowed for the non-invasive reconstruction of the white matter anatomy of the cerebral cortex human brain. Previous human tractography studies have used deterministic tractography, which creates clear reconstructed individual tracts based on the diffusion MRI data (Gong, He et al. 2009; Hagmann, Cammoun et al. 2008). However, deterministic tractography neither provides a mechanism for taking full account of the uncertainty in diffusion MR data, nor accounts for the possibility of crossing fibers within each voxel. For this study, we use an algorithm that runs numerous iterations (e.g. Monte Carlo simulation) of tract reconstructions using probabilistic tractography (Behrens, Woolrich et al. 2003; Behrens, Berg et al. 2007). Probabilistic tractography shows an improvement over deterministic

tractography as it takes into account the probability of crossing fibers during reconstruction. Every iteration of tract reconstruction will start at user-defined seed regions, which are three-dimensional voxel regions drawn in the same space as the subject's brain. For this study, once the gray/white matter boundary in the cerebral cortex of the brain is subdivided into smaller meaningful regions (i.e. a parcellation mask is derived), those regions can then be used all together as seed regions in network analysis. The goal of this study is to develop a method for deriving the reliable parcellation mask of the brain.

Reconstruction of Cortical Network in Chimpanzees

Just as important as mapping the human brain network, the examination of our closest living relative, the chimpanzee, could also provide important information regarding the key evolutionary similarities and differences between chimpanzees and humans. Recent studies in specific white matter tracts have elucidated key evolutionary differences and growth, such as the arcuate fasciculus, which has shown morphological changes in the connective nature of the functional regions related to speech (Rilling, Glasser et al. 2008). Fully understanding the architecture of the chimpanzee brain would provide insightful information regarding the similarities and differences in the organization of the brain networks between humans and chimpanzees. A cross-species comparison using the methods developed in this study, could help to reveal any important similarities or differences.

Even though recent studies have mapped the anatomical connectivity network of the human brain (Hagmann, Kurant et al. 2007; Hagmann, Cammoun et al. 2008; Bassett and Bullmore 2009; Gong, He et al. 2009; Honey, Sporns et al. 2009; Honey, Thivierge et al. 2010; Zalesky, Fornito et al. 2010), several key challenges have been demonstrated which would pose issues when attempting to map the chimpanzee neural network. First, obtaining the high quality diffusion MR data necessary for analysis in chimpanzees requires both dedicated MR scanner and chimpanzee subjects, which are not available at most research institutes. Second, the unique head anatomy (protruding jaws, small brain size) of chimpanzees requires overcoming many technical issues involved in scanning each subject. Third, as chimpanzee subjects are rare, a digital parcellation map for chimpanzees based on histological studies is non-existent.

Resources at the Biomedical Imaging Technology Center (BITC) and Yerkes National Primate Research Center at Emory University have laid the foundation for the development of this study. The large chimpanzee populations necessary for a species generalized study are available at Yerkes National Primate Research Center. BITC has access to a dedicated MR scanner, abundant human diffusion MR data, strong computational power (for tractography analysis), well-established protocols for both humans and chimpanzees, and experience in mapping the connectivity network in humans. Through utilization of these resources, we can overcome the obstacles mentioned previously and efficiently carry out the proposed study.

FUTURE APPLICATIONS ON AGING

Previous work in this area has shown much promise to reasonably deriving the connectivity network of the primate brain, even though it has been primarily in humans. Nevertheless, these past studies have demonstrated the need for improvements in the choice of tractography algorithm, quality of diffusion MRI data, and gray matter parcellation methods, which have been addressed in this study. With these foundational aspects in place, one specific aspect of the human brain that would benefit significantly from the application of this study is the array of adverse effects associated with aging. As the human brain sustains a lifetime of growth and damage, the white matter fibers change in shape and density, which reflect changes in cognitive functionality. Thus, the application of the understanding of these changes in the human connectivity network over the aging process could help to further elucidate the onset of many neurodegenerative diseases, such as Alzheimer's. Using the methods developed in this study, further applications in these areas will be possible.

The goal of this study is to develop a valid approach to deriving a cortical parcellation scheme for network analysis, independent of species anatomy.

CHAPTER 2: MATERIALS AND METHODS

1: MRI DATA ACQUISITION

At the Yerkes National Primate Research Center, 11 healthy chimpanzees (*pan troglodytes*) were scanned on a 3T Siemens Trio Scanner with a birdcage coil. T1 weighted anatomical images were collected with the following parameters: magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence, TR/TI/TE = 2400/1100/4.13 ms, flip angle = 8°, volume size = 256×256×154 mm, resolution = 0.8×0.8×0.8 mm3, 2 averages, total scan time = 20 minutes. Diffusion MRI data were acquired with the following parameters: TR/TE = 150/84ms, FOV = 130×230 mm, matrix size=72×128, 6/8 partial fourier, 8 averages for diffusion weighted images, 10 average for b_0 images, total scan time = 52 minutes.

2: IMAGE CORRECTION AND REGISTRATION

Both anatomical and diffusion MR data were analyzed using tools from the Oxford Center for Functional Magnetic Resonance Imaging of the Brain's Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl/). T1-weighted images were skull stripped, corrected for intensity bias, and noise reduction and contrast enhancement was applied. Diffusion MR data were first corrected for eddy current distortion, then in-house MATLAB (Matlab7, Mathworks) codes which were incorporated in statistics parametric mapping (SPM5, http://www.fil.ion.ucl.ac.uk/spm/) were used to correct susceptibility distortion based on the method of Andersson et al. (Andersson JL et al., 2003).

3.1: Node Definition (AAL)

Using the Automatic Anatomical Labeling (AAL) toolbox for SPM8 (http://www.cyceron.fr/web/aal_anatomical_automatic_labeling.html), a cortical segmentation map was produced for the 2mm MNI152 T1-weighted human template brain. Rigid-body linear transformations were performed from the chimpanzee template space to the MNI152 human template space. Next, non-linear registrations between the chimpanzee template space and human template space were carried out using each original affine transformation as a preliminary transformation. An inverse transformation matrix was used to apply the human AAL segmentation map onto the chimpanzee brain. Although the human AAL template in chimpanzee space was not used for the final brain parcellation scheme, the template map was used to help guide the further parcellation of the cortex.

3.2: Node Definition (Anatomically-Independent Parcellation (AIP))

In order for a gray matter parcellation scheme to be produced, a gray/white matter (GM/WM) interface mask was generated based on the partial volume values assigned by FAST algorithm in FSL. Voxels included in this interface region lie between the gray and white matter boundary so that the partial volume is greater than zero for each respective tissue type. Using the calculated interface mask, an algorithm was developed so that seed voxels are dynamically added such that the three-dimensional distance between the new seed and its nearest neighbor falls within 10% of a user-defined distance. Optimal distances were determined through experimental trials, presented in Table 1. The final seed voxels were used with three-dimensional region growing to segment the defined

number of cortical regions. Seeds were grown by randomly adding the neighboring 3x3x3 voxels to the seed, as long as they were un-marked gray matter voxels, iterating through the added voxels as center points until no more voxels were left for growing. Distributions of region volume after an initial growing round are provided in Figure 2. Multiple iterations of growing were performed, with the voxel closest to each patch's center of gravity as the new seed point for that patch. The grown regions were not directly related to any anatomical regions in the brain, as they were meant mainly for finding three-dimensional locations of hubs. Anatomical labeling of these regions was performed in post-processing.

4: GLOBAL TRACKING – NETWORK MAPPING

The procedure that would be implemented for determining the connectivity network from the parcellation scheme is summarized into the following five steps: first, estimate the voxelwise probability density function; second, determine the global connections to derive connectivity among different brain regions; third, count the thresholded tract volume as the index for the connectivity density between brain regions to the connection matrix; fourth, analyze them using graph theoretic-measures; fifth, using the probabilistic tractography algorithm in FSL's FDT toolbox (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html), run a set of analysis on each pair of regions to determine the densities of tracts and major hubs of the chimpanzee brain. This technique will be validated concurrently for human diffusion MRI data, alongside chimpanzee data.

CHAPTER 3: RESULTS

NODE DEFINITION (AAL)

The goal of this study was to formulate a reliable method for deriving the parcellation mask of any given primate brain, required for gross tractography analysis. Initially, the proposed parcellation of the chimpanzee brain included mapping the human Automatic Anatomical Labeling (AAL) template onto the chimpanzee brain by a non-linear transformation using FMRIB's Nonlinear Image Registration Tool (FNIRT) in the FSL toolbox (http://www.fmrib.ox.ac.uk/fsl/fnirt/index.html). Optimal parameters were determined by varying input sampling and blurring levels, and is provided in Appendix 1. However, due to multiple mismatches between the AAL parcellation borders and corresponding anatomical regions in the chimpanzee brain (Figure 1), this technique was not considered reliable for the gross parcellation of the primate brain.

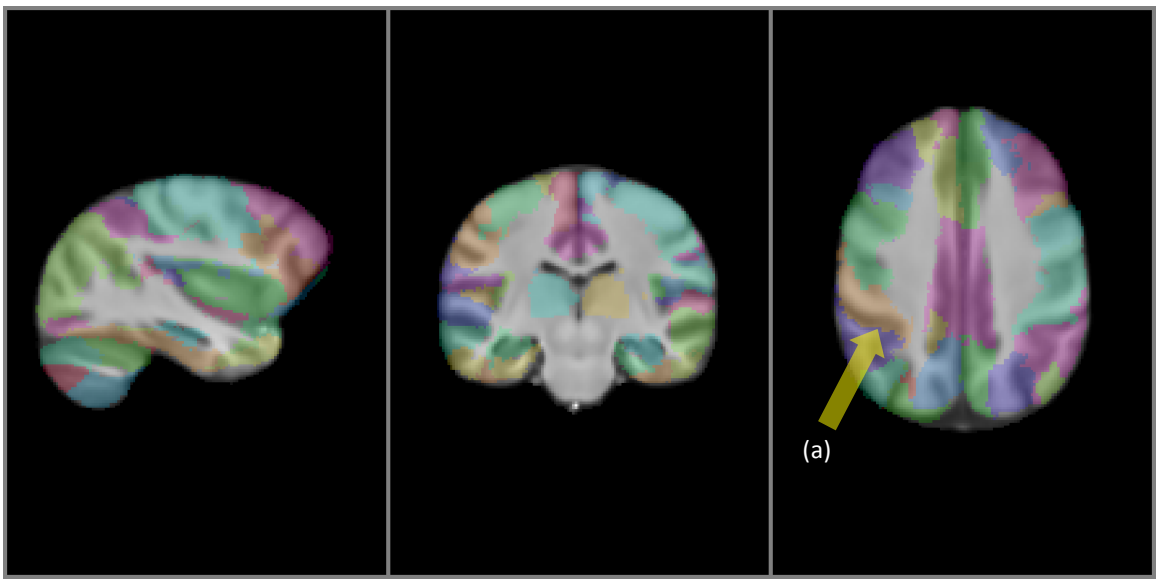

Figure 1. Parcellation of chimpanzee template brain using non-linearly transformed human AAL parcellation. It should be noted that there are obvious mismatches in cortical boundaries, (a) for example, as well as insufficient coverage of the cortical gray matter.

NODE DEFINITION (ANATOMICALLY-INDEPENDENT PARCELLATION (AIP))

The failure of the first method motivated the development of a new technique, independent of any anatomical labeling. The developed parcellation scheme considered reliable for tractography analysis includes the gray/white matter (GWM) boundary regions of the brain split into a user-defined number of nodal regions, which are similarly sized, shaped, and spaced out over the primate GWM boundary segmentation. The distances between the initial seed points were determined by trial and error and are presented in Table 1. Along with the iterative growing of the patches, standard deviations of patch volume distributions decrease over time (rate dependent on the number of nodes); however, eventually begin to rise after a number of iterations. Standard deviation curves are presented for 200, 500, 1000, and 3000 nodes in Figure 3. The final nodal parcellation patches have no direct anatomical relevance, since connectivity mapping

results will be interpreted for the strict spatial locations of the connectivity hubs in later analysis. Final parcellation maps are presented in Figure 4-6.

After normalized and averaged connectivity maps are generated for each subject, the observed connectivity hubs can be labeled anatomically by experts of each respective primate. For validation purposes, twenty iterations of the tractography analysis were run on each subject to determine an average connectivity network for each subject. By using multiple iterations with uniquely generated parcellation schemes, the inter-subject results hold more significance regarding the hub's spatial locations.

Table 1. Optimized Distances per Node Size (variability allowed = 1% of length)

Desired >	**200**	**500**	**1000**	**3000**
Seed Separation	14	9.6	7.14	4.55
Average Nodes	200.2	498.8	1000.4	2996.8
StDev Nodes	2.49	6.66	8.47	12.82

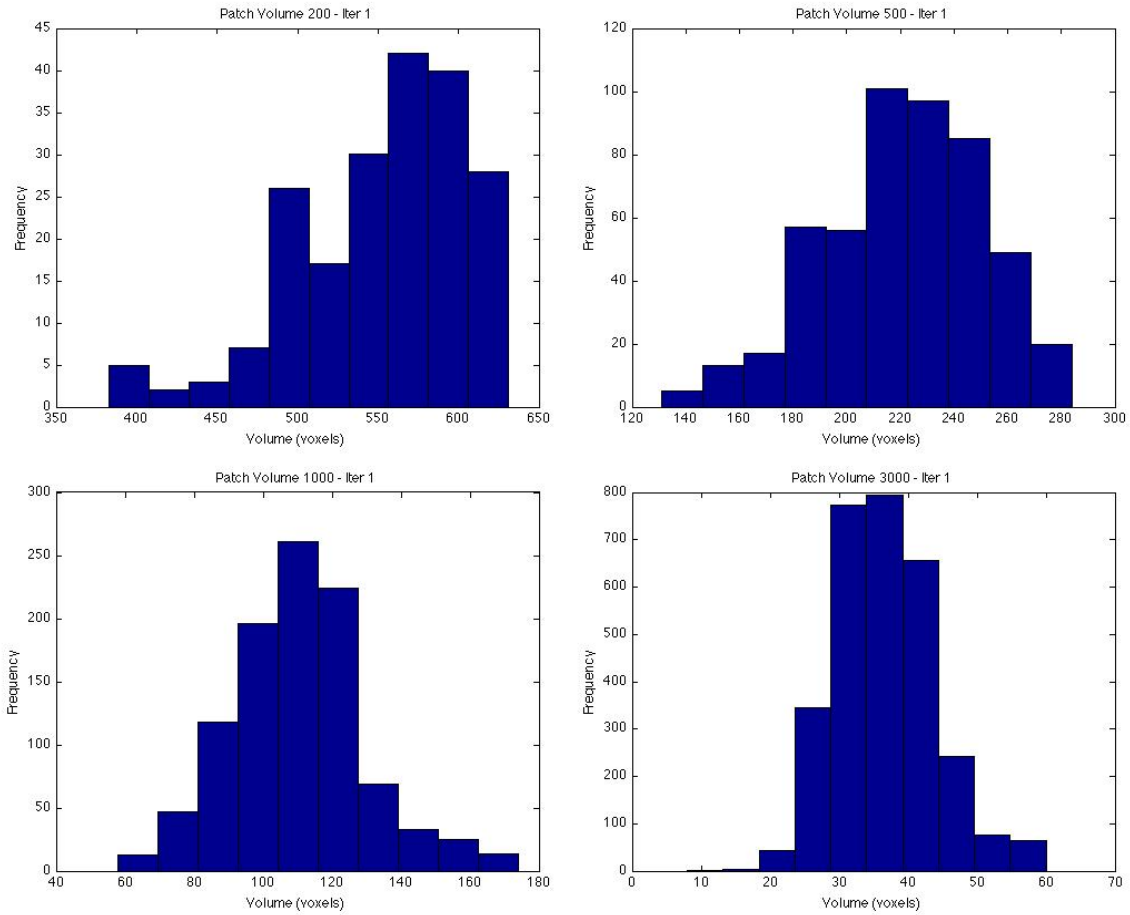

Figure 2. Distribution of Patch Volume with (from top-bottom, left-right) 200, 500, 1000, and 3000 nodes. Distributions are computed after first round of region growing; initial seed locations determined by dynamic addition.

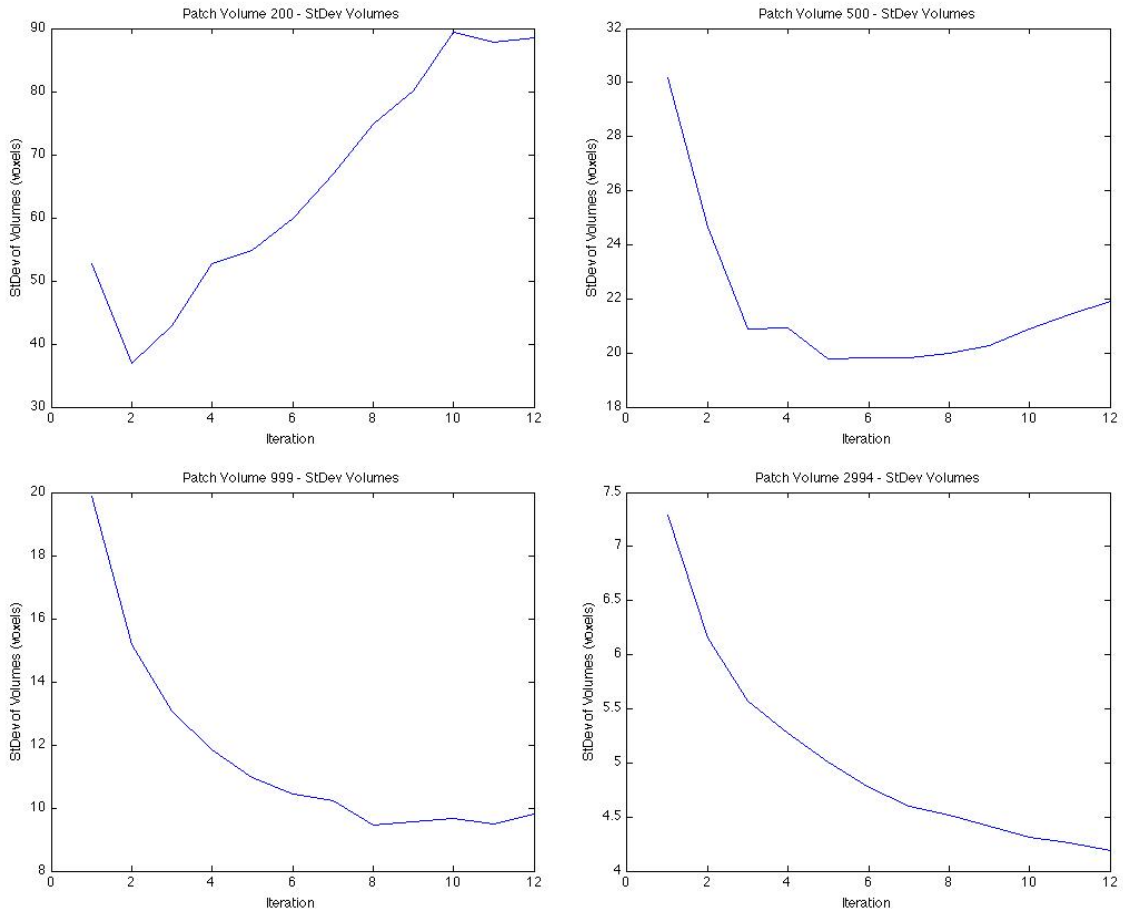

Figure 3. Distribution Width of Patch Volume with (from top-bottom, left-right) 200, 500, 1000, and 3000 nodes over 12 iterations of growing with center of gravity re-sampling of seed points.

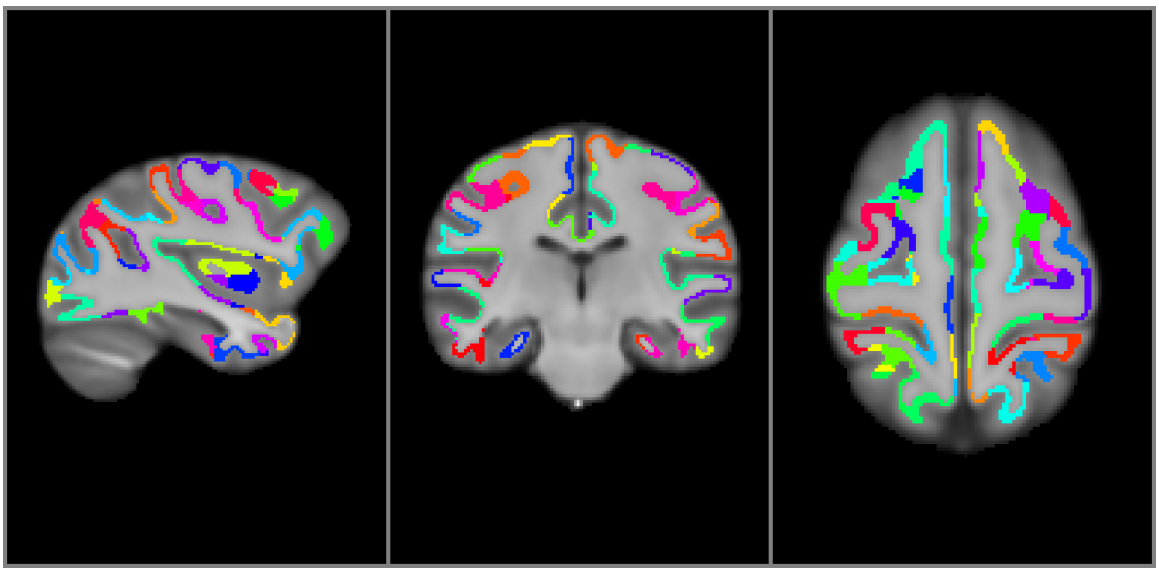

Figure 4. General parcellation of WGM boundary mask using 200 nodes, with sagittal (left), coronal (center), and axial (right) planes shown.

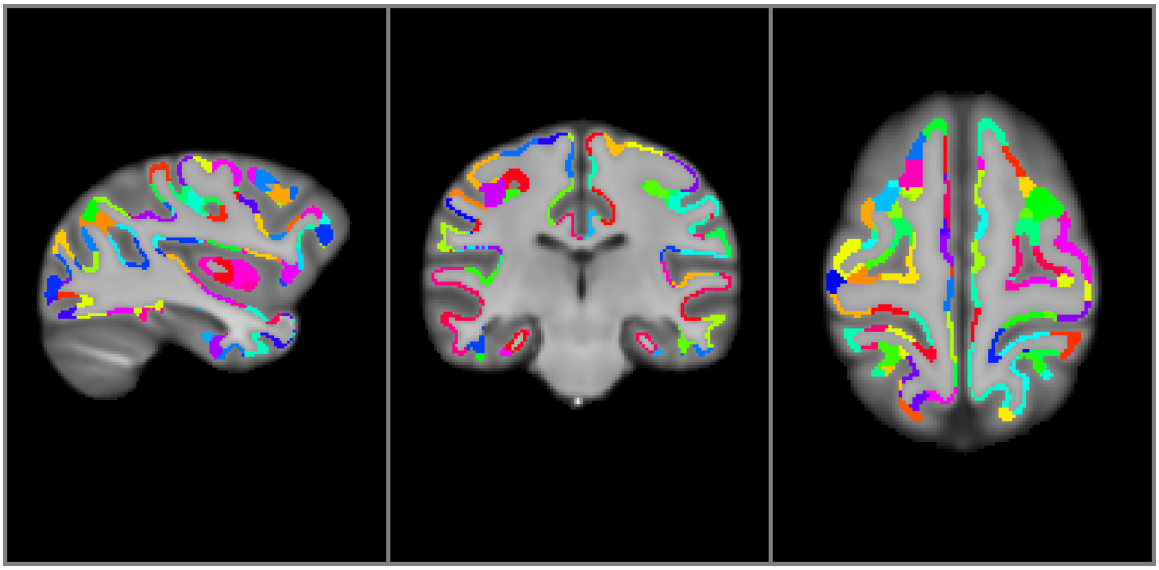

Figure 5. General parcellation of WGM boundary mask using 500 nodes, with sagittal (left), coronal (center), and axial (right) planes shown.

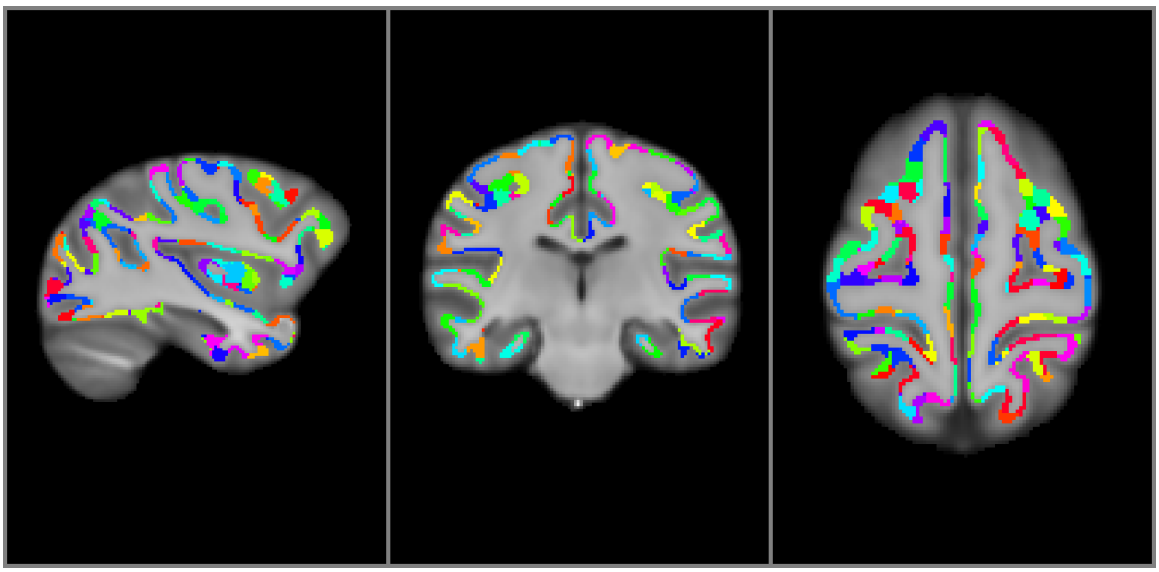

Figure 6. General parcellation of WGM boundary mask using 1000 nodes, with sagittal (left), coronal (center), and axial (right) planes shown.

CHAPTER 4: DISCUSSION

The technique developed for cortical parcellation shows promise regarding further applications on primate connectivity architecture reconstruction. The initial parcellation scheme was dependent on the non-linear transformation between the human and chimpanzee brain templates; however, obvious structural and functional mismatches between several regions in each respective brain were observed. This finding was the motivation for the proposed parcellation scheme, which has anatomical definition independence. The presented method, involving dynamic node placement and region growing, allows for this technique to be applied over many different subjects and/or species. There is an increase in the resolution of the map since thousands of nodes can be defined, compared to an original 116 in the AAL template. The coverage of the gray/white matter boundary mask is also significantly improved as the AIP method is performed directly on the boundary mask, ensuring that all voxels are labeled. Finally, regarding the distribution of patch sizes, with the exception of 200 nodes, since the standard deviation of patch sizes were all approximately 10% of the mean patch size at the optimized iteration, the parcellation schemes' reliability was reassured. For Further analysis using each patch as a respective seed region, probabilistic tractography can be performed to quantify connections between each region, thus producing an overall connectivity map. This map can then be analyzed using graph theory to reveal structural hubs in the cerebral cortex. Post-analysis anatomical classifications on the structural hubs can reveal similarities and differences between different primates in cross-species comparisons. Furthermore, with the re-acquisition of each respective primate's data at a

more developed age, aging studies can reveal evolutionary differences in the development or degeneration of white matter connectivity.

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APPENDIX A. OPTIMIZED PARAMETERS FOR FNIRT HUMAN TO CHIMPANZEE TRANSFORMATIONS

Parameters / Files >	Chimp_2_T1NReg2	Chimp_2_T1NReg8
imprefm	1	1
impinm	1	1
imprefval	0	0
impinval	0	0
subsamp	4,2,1,1	4,2,1,1
miter	5,5,5,10	5,5,5,10
infwhm	8,4,2,0	8,4,2,0
reffwhm	8,4,2,0	8,4,2,0
lambda	300,100,75,30	300,100,75,30
estint	1,1,1,0	1,1,1,0
applyrefmask	1,1,1,1	1,1,1,1
applyinmask	1	1
warpres	8,8,8	8,8,8
ssqlambda	1	1
regmod	bending_energy	bending_energy
intmod	global_non_linear_with_bias	global_non_linear_with_bias
intorder	5	5
biasres	50,50,50	50,50,50
biaslambda	10000	10000
refderiv	0	0
cout	Reg2_cout	Reg8_cout
iout	Reg2_iout	Reg8_iout
fout	Reg2_fout	Reg8_fout
refout	Reg2_refout	Reg8_refout
Σ (Residuals)^2	5.7449E+05	5.7449E+05